

DEVELOPMENT OF A LOW COST IN-HOUSE METHOD FOR THE ANALYSIS OF HUMAN Y-CHROMOSOMAL MINI STR LOCI DYS19, DYS390 and DYS388

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INTRODUCTION

It is well known in the Forensic field that Short Tandem Repeat (STR) DNA on the human genome is one of the powerful markers for accurate identification of a criminal from an evidentiary sample. In sexual assault of a female individual by a male, the evidentiary sample submitted for DNA based identification of the male perpetrator is often a swab containing semen taken from the vagina or anus of the victim. However in most instances the vaginal or anal swabs consist of mixed DNA from the female victim and the male perpetrator. Depending on how the area was swabbed, the samples could contain a large proportion of female component which will eventually masks the DNA profile of the male perpetrator. In such situations accurate identification of the male profile is often not achieved (Cerri *et al.*, 2003). Analyzing DNA markers on the Y chromosome will target only the male DNA component containing in the swab making accurate identification of male assailant feasible.

If such a mixed evidentiary material contain partially degraded male DNA, identification of the male assailant is even more challenging because of fragmentation of the Y-chromosomal DNA due to degradation, which could result in the failure of PCR amplification of the full length of the Y-chromosomal STR region of interest. Therefore Y STRs that make shorter amplicons in PCR (Y-mini STR) are a reliable way to analyse partially degraded male DNA (Park *et al.*, 2007; Asamura *et al.*, 2007).

Commercially available kits such as the Promega power plex Y STR (Promega, USA) are costly, and would render the test unaffordable in Sri Lankan situation. Further this method needs an Automated DNA Genetic analyzer to genotype the Y-STR markers. However genotyping using a manual silver staining detection system is a relatively rapid, inexpensive alternative to automated genotyping techniques (Benbouza *et al.*, 2006). Therefore the development of a low

cost genotyping method for Y-STRs based on silver staining detection method to analyze partially degraded male DNA is crucial to make the identification of the male assailant using sexual assault swabs containing partially degraded male DNA.

The objective of this study was to develop a low cost in-house method to analyse short amplicons of Y-STR DNA markers for the DYS19, DYS388 and DYS390 loci.

MATERIALS AND METHODS

The first stage of the study was to develop in-house Y STR standard size markers (standard allelic markers) for DYS19, DYS388 and DYS390 loci. Nine male DNA samples that can contribute the maximum no of polymorphic alleles to each locus were selected. The selected male samples (of which the alleles were known by validating against a Promega PowerPlex Y STR kit) were pooled to construct allelic ladders. DNA extractions were done using a genomic DNA extraction kit (Geneshun, China). 5 µL of the DNA mixture was used for PCR in a total reaction volume of 50 µL. The PCR reaction consisted of;

- 10 X STR buffer 5 µL
- 10 mM dNTP 5 µL
- 2.5 µM STR Primer (F) 2.5 µL
- 2.5 µM STR primer (R) 2.5 µL
- 5 u/µL Taq DNA polymerase 0.2 µL
- Sterile Distilled water 29.8 µL
- DNA Template 5µL

The PCR reaction was carried out in a GeneAmp 9600 (Applied Biosystem, USA) thermal cycler under the following PCR protocol: 96 °C for 2 min; 38 cycles of 94 °C for 20 s, 58 °C for 45 s, 72 °C for 90 s; a final extension at 72 °C for 7min. Subsequent to PCR, the products were subjected to 3% w/v Agarose gel electrophoresis (*Figure:1*) to verify the success of the PCR reaction. The samples amplified in the 1st PCR reaction were

then subjected to 4% denaturing Polyacrylamide gel electrophoresis (*Figure:2*).

The Primers were newly designed (Table 1) targeting the three polymorphic loci DYS19, DYS388 and DYS390 on the Y chromosome in order to reduce the amplicon length using the Primer3 software and electronic PCR (Abd-Elsalam, 2003). Using the Y-STR ladders (developed in the 1st stage of this study) and custom primers, Y mini STR ladders were developed (*Figure:3*) and evaluated against male samples of which the alleles had been analyzed by Promega PowerPlex Y STR kit. PCR reaction components for Y mini STR amplification were as same as the full length Y-STR amplification except the template DNA. 2 µL of PCR products of the Y-STR ladders were used as DNA template to re-amplify Y Mini STR ladders. PCR conditions were also similar to Y-STR amplification, only the annealing temperature of the PCR protocol was increased from 57^oC to 58^oC to increase the specificity of primer binding. 2.5 µl of each PCR products was ran in 4% denaturing PAGE (*Figure:4*) along with a male positive control amplicon of which the allelic size was known from Promega Power Plex Y-STR system.

RESULTS

Newly make custom Y-STR ladders were visualized by 3% agarose gel electrophoresis followed by 4% polyacrylamide gel electrophoresis (*Figure:1 & 2*). Then primers were newly designed to reduce the amplicon length (*Table:1*). Subsequently the Y mini STR ladders were developed with the custom made primers (*Figure:3*) and analyzed against degraded male DNA (*Figure:4*).

DISCUSSION

The proportion of the amplicon size reduction achieved by the present study compared to conventional Y-STR systems for DYS19, DYS388 and DYS390 were 41%, 24% and 21% respectively (*Table: 2*). The allelic ladders constructed for each of the three systems enabled their use as size markers in polyacrylamide based analysis of Y-STR mini loci. The integration of these systems to the silver staining procedure enabled their analysis at significantly lower cost (50%) compared to a commercially available kit. The findings of the present study would be an initial step towards developing a system of which the entire Y-chromosomal STR test panel that

consist of 12 loci will be analyzed using a single low cost methodology in Sri Lanka in the future.

CONCLUSION

Y- Chromosome mini STR systems developed herein make significantly reduced amplicon sizes of the three Y-STR markers analyzed and thereby enabling the analysis of a post coital samples of sexual assault containing degraded male DNA. Custom made primers and allelic ladders of the present study can be used to analyse Y chromosome mini STR system consisting of the three loci DYS19, DYS388 and DYS390 cost effectively using a polyacrylamide based silver staining procedure.

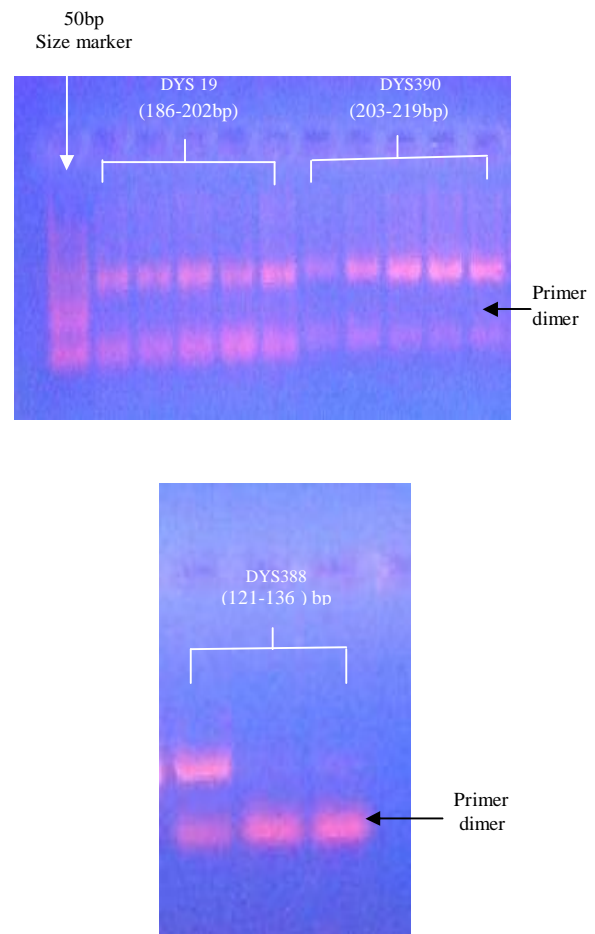


Figure 1: Agarose Gel electrophoresis of DYS19, DYS390 and DYS388 Y STR standard size markers.

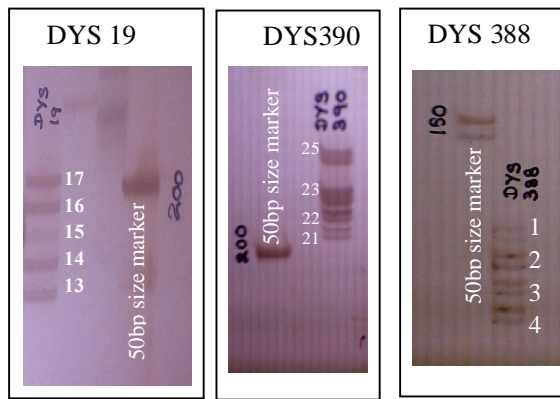


Figure 2: Polyacrylamide (4%) gel electrophoresis of DYS19, DYS390 and DYS388 conventional Y STR amplicons with the standard size markers.

Y Chromosome Loci	Custom Y chromosome Mini Primers For the current study (Forward and Reverse)	Annealing temp. °C
DYS19	3' TGGTCTTCTACTTGTGTCAATAC AGA 5' 3' AAAATGAGGTATGTCTCATAGA AAAGA 5'	59.30 58.05
DYS388	3' TCATGTGAGTTAGCCGTTTAGC 5' 3' AGCGAGAGTCCGTCCTCAAAT 5'	59.43 59.04
DYS390	3' CTGCATTTTGGTACCCATA 5' 3' GCAATGTGTATACTCAGAAACA AGG 5'	58.36 59.17

Table 1: Details of Y chromosome mini STR primers designed for the present study.

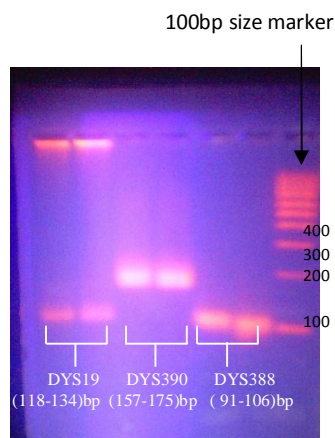


Figure 3: Agarose Gel electrophoresis of DYS19, DYS388, and DYS390 Y chromosome mini STR ladders

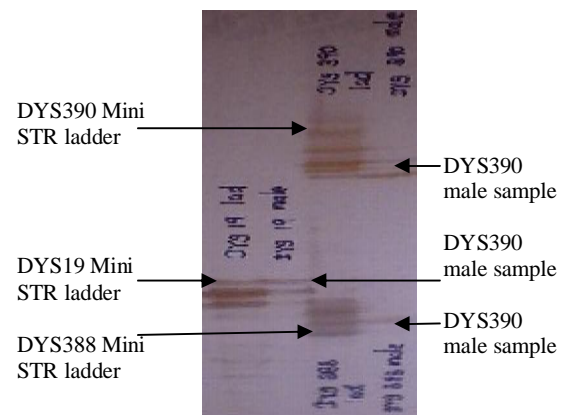


Figure 4: Polyacrylamide (4%) gel electrophoresis of PCR amplicons of DYS19, DYS388 and DYS390 Y Chromosome Mini STR ladders

Y chromosome Loci	Y STR amplicon Size (bp)	Y mini STR amplicon size	Band Size Reduction as a percentage
DYS19	182-206	114-138	37%
DYS388	121-136	91-106	25%
DYS390	195- 227	151-183	23%

Table 2: Proportion of the amplicon size reduction of Y chromosome mini STR primers designed for the present study with compared to Y STR system.

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